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Population dynamics of *Fusarium oxysporum* f.sp. *niveum* and *F. solani* f.sp. *cucurbitae* in commercial watermelon fields in Tunisia

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Experiments were conducted in field plots to evaluate the level and the dynamic population of *Fusarium* species propagules of in three commercial watermelon fields in Tunisia surveyed two successives years. Populations of *Fusarium oxysporum* f. sp. *niveum* and *F. solani* f. sp. *cucurbitae* in naturally infested fields of watermelon were enumerated by soil dilution method and subsequent pathogenicity tests. The results showed that *Fsc* colonies number recovered from the field soils was higher than the value of *Fon* colonies, in each field and for the two years. Before planting, the density of *Fsc* propagules in the field soils was higher than the number of *Fon* colonies, in each field and for the two years. It varies from 193 to 128 propagules/g soil for the 1st year and between 986 to 568 propagules/g soil in the 2nd year for field II. The highest inoculum density of *Fsc* was found at Field II (986.67±172.09 CFU g^{-1} soil) during the 2nd year, before plantation. Pathogenicity test revealed that for pathogens isolated from the deep 20-30 cm, the number of pathogenic colonies is relatively lower and counted between 40 to 70%.

Fusarium wilt and Fusarium root and collar rot of watermelon (*Citrullus lanatus* Matsu & Nakai) caused by *Fusarium oxysporum* f.sp. *niveum* (WC. Snyder & H.N. Hans.) and *F. solani* f. sp. *cucurbitae* (*Fsc*) respectively, are the most important soil-borne diseases affecting this cucurbit in Tunisia (Boughalleb and El Mahjoub, 2005; Boughalleb et al., 2005). However, little is known about the range and distribution of inoculum densities of these pathogens in infested commercial fields used for watermelon production in Tunisia as well as other areas of the world. In Israel, Netzer (1976) demonstrated that the population of *Fon* within a single heavy infested watermelon field ranged between 400 and 1400 CFU/g of soil. Within this inoculum level, more than 95% of watermelon cv. Sugar Baby seedlings of the wilted in a greenhouse bioassay. In Florida, Hopkins and Elmstrom (1984) reported that the populations of *F. oxysporum* averaged around 1500 CFU/g of soil in most watermelon research crop. Zhou and Everts (2003) studied the inoculum densities of *F. oxysporum* f.sp. *niveum* in watermelon commercial fields in Maryland and Delaware, they found that the inoculum densities of this fungus ranged from 100 to 1200 CFU/g of soil in 76% of surveyed fields. Watermelon cv. Sugar Baby was planted in samples of soil collected from these fields showed more than 20% of

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infected plants. They demonstrated also that the ratio of pathogenic *F. oxysporum* linearly increased with inoculum density of *F. oxysporum* f.sp. *niveum*. In Tunisia, Boughalleb et El Mahjoub (2006), the estimated inoculum densities required to cause 50% of wilted plants on cv. Giza (DI_{50}) was respectively 670 and 171 $CFUg^{-1}$ of soil for *F. solani* f. sp. *cucurbitae* and *F. oxysporum* f. sp. *niveum*.

The objective of this study was to survey of the dynamic population of *Fon* and *Fsc* in commercial watermelon fields in Tunisia and to estimate the population of these pathogens by pathogenecity tests of the selected colonies.

Materials and Methods

Field survey

Three commercial watermelon fields from the main watermelon production areas of Tunisia were surveyed during two years to evaluate the inoculum density of *Fon* and *Fsc*.

Soil sampling and isolation of *Fon* and *Fsc*

To quantify the inoculum density of *Fon* and *Fsc* in commercial watermelon fields, 20 soil samples were collected from each field at two times: before plantation and at harvest, during two years from three watermelon fields at three soil deep, where symptomatic watermelon plants were sampled.

For each soil sample, approximately 500g of soil was collected from a depth 10-30cm. All soil samples were stored at 4°C until used. Soil samples from the same field and same deep were combined into one sample and mixed thoroughly. The population of *Fon* and *Fsc* in each soil sample and depth was determined by plating aliquots of a dilution series of the soil. A 15g sub-sample of soil was added to 90 ml of 0.1% autoclaved water agar. The resulting soil suspension was mixed for 5 min using a magnetic stirrer. 1 ml of this suspension was plated onto each ten petri plates of Komada's medium (Komada, 1975). Three replicates of soil dilutions were prepared for each field sampled. All plates were incubated at 25°C for 4-5 days, and the total number of colonies of *F. oxysporum* and *F. solani* was counted. Colonies were identified as *F. solani* or *F. oxysporum* according to morphological criteria indicated by Booth (1971) and Nelson et al. (1983).

Pathogenecity test from each sample and soil deep

The ratio of pathogenic to non-pathogenic colonies of *Fon* and *Fsc* for each field sampled was determined by virulence tests of random, representative colonies. Ten colonies were selected for pathogenecity tests from each sample and soil deep. The pathogenicity of all selected isolates was assessed on watermelon seedlings of the susceptible cv. Sugar Baby.

The inoculation method used for isolates of *Fon* and *Fsc* was as described by Boughalleb and El-Mahjoub (2006). All plants were maintained in a greenhouse conditions, seedlings were watered and fertilized as needed to maintain normal growth. The symptoms were assessed weekly for 6 weeks after seeding. A fungal isolate was regarded as pathogenic if at least one seedling per cell developed symptoms typical of Fusarium wilt or Fusarium root and collar rot

Data analysis

Were performed by analysis of variance using the software SPSS program and mean comparisons were made using the LSD test ($P = 0.05$).

Results

Fusarium isolation and identification

Different types of Fusarium colonies developed on Komada medium. Colonies of *Fsc* and *Fon* differed from other *Fusarium* species in colour and morphology and were confirmed by microscope identification. Their growth on PDA was rapid, with abundant aerial mycelium. Microscopic identification showed that microconidia were formed in false heads on long

branched monophialides (*Fsc*) or on short branched monophialides (*Fon*) and in the presence of abundant chlamydospores (Nelson et al., 1983).

Fungus population in field soil

The number of *Fsc* colonies that were recovered from the field soils was higher than the number of *Fon* colonies (Tables 1, 2 and 3), in each field and for the two years.

Table 1. Number of colonies of *Fusarium solani* and *F. oxysporum* recovered from the soil of field I surveyed for two years and percentage of pathogenic colonies.

Soil deep (cm)	*Fusarium solani*				*Fusarium oxysporum*			
	First year		Second year		First year		Second year	
	1*	2**	1	2	1	2	1	2
Number of colonies								
0-10	755.56[a]±104.11[b]	720.00±156.2	906.67±180.24	486.67±168.26	428.89±679.42	33.33±17.64	24.44±13.88	2.22±3.85
10-20	660.00±187.57	353.33±57.74	871.11±193.37	891.11±16.78	204.44±336.93	35.55±23.41	22.22±21.43	22.22±21.43
20-30	631.11±161.43	284.44±7.70	477.77±93.41	648.89±66.78	104.45±157.95	20.00±17.64	17.78±16.78	35.55±20.37
Percentage of pathogenic colonies[c]								
0-10	60	80	50	70	50	90	50	90
10-20	70	70	70	70	60	90	70	80
20-30	40	70	50	40	40	70	40	50

* Before plantation, ** after plantation

a: Mean of three replicates and for each replicate ten plates were counted for each soil sample

b: Standard error

c: Ten colonies were used for pathogenicity tests from each sample and soil deep.

In the samples taken from the top 30-cm soil layer during two years at two differentes times, the population of the fungus for *F. solani*, before planting, was found to vary from 755 to 631 propagules/g soil in the 1st year and between 906 to 486 propagules/g soil in the 2nd year for field I (Table 1). It varies from 193 to 128 propagules/g soil for the 1st year and between 986 to 568 propagules/g soil in the 2nd year for field II (Table 2). For field III it varies from 160 to 116 propagules/g soil in the 1st year and from 175 to 82 propagules/g soil (Table 3). The highest inoculum density of Fsc was found at Field IV (986.67±172.09 CFU g^{-1} soil) during the 2nd year, before plantation (Table 2)

The results of the population of the fungus *F. oxysporum* in each field, were with lower inoculum density than that of *Fsc*, and are indicated in Tables 1, 2 and 3. In most of the fields, the inoculum density was between zero and 8 CFU g^{-1} soil during the 1st year. In the field I, the inoculum density of *Fon* varied between 20 to 428 propagules/g of soil for the 1st year and varied between 2 to 35 propagules/g of soil during the 2nd year. The highest inoculum density of *Fon* was found at Field II, in the 2nd year, before plantation (606.67±342.80 CFU g^{-1} soil) (Table 2).

Pathogenic colonies from each sample and soil deep

Identification of pathogenic isolates of *Fsc* and *Fon* was done according to the symptoms observed respectively: crown and root rot, and wilt. Most of the selected *Fsc* colonies were pathogenic to watermelon seedlings cv. Sugar Baby (Tables 1, 2 and 3), the same occurred for *Fon* colonies but with less percentage in comparison with *Fsc*. It revealed that for colonies decovered from 0-10 cm deep, pathogenic colonies percent vary between 50 and 90. For 10-20 cm deep, the values ranged from 60 to 90%. In case of pathogens isolated from the deep 20-30 cm, the number of pathogenic colonies is relatively lower and counted between 40 to 70%

Discussion

The inoculum density, in the fields sampled, of *Fon* and *Fsc* were determined. The study showed that inoculum of *Fsc* was more prevalent than that of *Fon*. Our results revealed that the inoculum densities of *Fsc* and *Fon* in Tunisian watermelon fields varied respectively from 280 to 906 CFU g^{-1} soil and from 20 to 480 CFU g^{-1} soil. These densities are lower than those found in other reports; Netzer (1976) showed a range of *F. oxysporum* from 400 to 1400 CFU / g of soil in heavely infected fields cropped with watermelon cv. Sugar baby. In Maryland and Delware watermelon fields, inoculum density of *Fon* were higher ranging from 100 to 1200 CFU / g of soil (Zhou and Everts, 2003).

Table 2. Number of colonies of *Fusarium solani* and *F. oxysporum* recovered from the soil of field II surveyed for two years and percentage of pathogenic colonies

Soil deep (cm)	*Fusarium solani*				*Fusarium oxysporum*			
	First year		Second year		First year		Second year	
	1	2	1	2	1	2	1	2
Number of colonies								
0-10	128.89[a]±107.98[b]	268.89±61.94	986.67±172.09	82.22±26.95	6.67±6.67	4.45±3.85	20.00±2.64	0.00±0.00
10-20	193.33±196.75	164.44±10.18	824.44±196.07	91.11±13.88	0.00±0.00	4.45±3.85	606.67±34.28	8.88±3.85
20-30	137.78±34.21	108.89±19.24	568.89±157.53	20.00±13.34	6.67±11.55	4.45±3.85	602.22±28.67	0.00±0.00
Percentage of pathogenic colonies[c]								
0-10	50	60	70	80	90	100	80	0
10-20	80	70	80	90	0	100	70	100
20-30	30	40	50	30	80	100	60	0

a: Mean of three replicates and for each replicate ten plates were counted for each soil sample
b: Standard error
c: Ten colonies were used for pathogenicity tests from each sample and soil deep.

Table 3. Number of colonies of *Fusarium solani* and *F. oxysporum* recovered from the soil of field III surveyed for two years and percentage of pathogenic colonies

Soil deep (cm)	*Fusarium solani*				*Fusarium oxysporum*			
	First year		Second year		First year		Second year	
	1	2	1	2	1	2	1	2
Number of colonies								
0-10	116.94[a]±49.05[b]	160.00±6.67	175.55±23.41	55.56±10.18	00.00±0.00	8.89±10.18	6.66±6.67	8.88±3.85
10-20	160.00±86.67	162.22±42.86	95.56±39.06	66.67±11.55	2.22±3.85	6.67±11.55	2.22±3.85	8.88±3.85
20-30	144.44±44.39	140.00±40.00	82.22±78.97	75.56±25.24	2.22±3.85	00.00±0.00	2.22±3.85	2.22±3.85
Percentage of pathogenic colonies[c]								
0-10	90	80	60	80	0	90	80	90
10-20	90	90	70	80	100	100	100	100
20-30	70	50	40	30	0	0	60	70

a: Mean of three replicates and for each replicate ten plates were counted for each soil sample
b: Standard error
c: Ten colonies were used for pathogenicity tests from each sample and soil deep.

Boughalleb and El Mahjoub (2006) showed a range of *F. oxysporum* from zero to 200 CFU / g of soil and that the incidence of the disease was significantly correlated with the inoculum density. However in other reports, it was indicated that levels of F.

oxysporum in field soils may not correlate with incidence of Fusarium wilt (Hopkins and Elmstrom, 1984). Pathogenecity tests showed that most of the *Fsc* and *Fon* isolates were pathogenic to watermelon seedlings.

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